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Tuber respiratory profiles during low temperature sweetening (LTS) and reconditioning of LTS-resistant and susceptible potato (*Solanum tuberosum* L.) cultivars

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ABSTRACT

Potato cultivars Premier Russet, GemStar Russet, Defender and Russet Burbank differ substantially in resistance to low temperature sweetening (LTS) and associated metabolism. 'Gemstar Russet' and 'Premier Russet' have moderate and high resistances, respectively, while 'Defender' loses processing quality progressively during storage at 9 °C, and similar to 'Russet Burbank', has virtually no resistance to LTS at 4 °C. The different mechanisms of LTS resistance or susceptibility in these cultivars were indicated by changes in sucrose (Suc), fructose (Fru) and glucose (Glc) concentrations in relation to tuber respiratory profiles during wound healing (9 °C), LTS (4 °C) and reconditioning (16 °C). At 4 °C, 'Premier Russet' tubers maintained low levels of Suc and reducing sugars (RS, Glc + Fru), while 'GemStar Russet' tubers accumulated Suc with little inversion to RS. 'Defender' and 'Russet Burbank' tubers accumulated RS during LTS but only moderate levels of Suc. Changes in RS content reflected the combined activities of acid invertase and its endogenous inhibitor. In response to an immediate drop from 9 °C to 4 °C, tuber respiration decreased to a minimum and then increased to a new maximum over the next approximately 5 days, before decreasing to a constant basal rate at 4 °C. Relative changes in respiration from the minimum to maximum rate during cold acclimation (respiratory acclimation response, RAR) were 80% for 'GemStar Russet' and 'Defender', 51% for 'Russet Burbank' and 26% for 'Premier Russet'. The RARs correlated with total sugar (Suc + Glc + Fru) accumulation during LTS and likely reflected the metabolic energy required to catabolize starch to Suc, Glc and Fru. The relative ratio of Fru/Glc was also demonstrative of LTS-resistance, discriminating genotypes that accumulated Suc versus RS under LTS conditions. Changes in carbohydrates, invertase, respiration rates and RARs in response to temperature over the wound healing, LTS and reconditioning phases of storage characterized the LTS phenotypes unique to each cultivar, and revealed different mechanisms of resistance to LTS.

1. Introduction

Potato production in the Pacific Northwest (Washington, Idaho and Oregon) is dominated by long season russets utilized in processing finished fries for the quick service restaurant industry. The ability to store tubers long-term (>6 months) can improve grower returns by 30–60% (NASS, 2012) in addition to contract incentives based on low reducing sugar (RS; glucose + fructose) content at the time of sale. While storage of potatoes at lower than normal temperatures (<9 °C) can extend marketability, minimize disease pressure and postpone sprouting, many cultivars are susceptible to cold-induced Suc and RS accumulation, known as low temperature sweetening (LTS; Workman et al., 1979; Richardson et al., 1990; Zrenner et al., 1996; Matsuuro-Endo et al., 2004). Glucose (Glc) and fructose (Fru) react with free amino acids during processing (Maillard reaction), resulting in unacceptable darkening of finished fries (Shallenberger et al., 1959; Driskill et al., 2007) and increased formation of the animal carcinogen acrylamide (Bethke and Bussan, 2013).

Relative resistance or susceptibility to LTS can vary dramatically among cultivars with respect to carbohydrate and respiratory metabolism. Critical control points in the LTS pathway have been identified through detailed metabolic studies (Barichello et al., 1990; Zrenner et al., 1996; Sowokinos et al., 1997; McKenzie et al., 2013) and molecular mapping (Sowokinos et al., 1997; Menendez et al., 2002; Li et al., 2008). In general, LTS is characterized by the cold-induced catabolism of starch (Isherwood, 1976), synthesis of Suc (Viola et al., 1991; Hill et al., 1996) and its subsequent hydrolysis by acid invertase, resulting in RS accumulation and the associated deterioration of process quality (Pressey, 1969; Zrenner et al., 1996;

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Bhaskar et al., 2010). As carbohydrate metabolism is a predominant sink for ATP in stored potatoes, changes in respiration in response to low temperature storage can be indicative of LTS (Isherwood, 1973). Whole tuber respiration undergoes a predictable respiratory acclimation response (RAR) to a rapid drop in storage temperature (Isherwood, 1973, 1976; Schippers, 1977; Workman et al., 1979). Low oxygen storage (<3%) can mitigate LTS and correspondingly quench the RAR in cold-stored tubers, although the precise response is cultivar dependent (Harkett, 1971; Sherman and Ewing, 1983; Zhou and Solomos, 1998).

To date, studies to characterize the significance of the RAR have focused solely on sweetening susceptible cultivars (Isherwood, 1973, 1976; Amir et al., 1977). Four cultivars representing a range of LTS-resistant phenotypes were selected from the Northwest Potato Variety Development program to determine the relationships between RAR and carbohydrate changes during sweetening and reconditioning. 'Premier Russet' is highly resistant to LTS, 'GemStar Russet' is moderately resistant, 'Russet Burbank' is moderately susceptible and 'Defender' is highly susceptible. Following LTS in both long- and short-term storage studies, reconditioning potential was evaluated for each cultivar by subjecting the tubers to 3 weeks of storage at 16 °C (95% RH). Changes in carbohydrates, invertase and inhibitor activities, respiration rates and RARs in response to temperature changes during the wound healing (9 or 12 °C), LTS (4 °C) and reconditioning (16 °C) phases of storage characterized the distinct LTS phenotype for each cultivar and revealed different mechanisms of resistance or susceptibility to LTS.

2. Materials and methods

2.1. Plant materials

Potato (*Solanum tuberosum* L.) tubers for the postharvest studies were grown at the Washington State University Irrigated Research and Extension Unit at Othello, WA (46°47.277′ N. Lat., 119°2.680′ W. Long.). Certified (G3) seed tubers of each cultivar (Russet Burbank, Defender, GemStar Russet, Premier Russet) were obtained from local growers in the fall and stored at 4 °C (95% RH) until planting. Seed-tubers were cut into 50–64 g seedpieces, suberized for 3–5 days at 9 °C (95% RH) and planted in mid-April each year of study (2005–08). Tubers were grown with irrigation according to commercial recommendations for late season long russet cultivars in the Columbia Basin, as described by Driskill et al. (2007). Vines were removed using a flail-type mower ca. 155–158 days after planting (DAP) to allow 14–21 days for tuber maturation before harvest. Tubers were harvested with a single-row mechanical harvester 169–176 DAP, washed, sorted by weight, and unless specified otherwise the 171–284 g tubers were used for postharvest studies.

2.2. Long-term storage studies

The effects of long-term low temperature storage on process quality, tuber sugar content and reconditioning ability were assessed over three storage seasons (2005/06, 06/07 and 07/08) for cultivars Defender, GemStar Russet and Premier Russet. Tubers were initially wound-healed for 11 days at 12 °C (95% RH) and then stored at 4 °C or 9 °C (control) for an additional 217 days. The tubers were sampled at 11, 44, 112, 169, 228 and 250 days after harvest (DAH) each year of study for Suc and RS (Glc + Fru) analyses as described in Knowles et al. (2009). Reconditioning potential was determined after 228 days of storage by placing samples of tubers at 16 °C (95% RH) for an additional 22 days (from 228 to 250 DAH) to determine the extent to which RS could be lowered and process quality improved. Sprouting over the 250 day storage period was inhibited by treating tubers with 0.75 mmol kg^{-1} 3-nonen-2-one (Bedoukian Reasearch Inc., Danbury, CT) as needed (Knowles and Knowles, 2012). Four replicates of three tubers per treatment were analyzed on each sampling date ($n = 36$ over the 3 year study period).

2.3. Short-term storage studies

Shorter term storage studies were also conducted to profile the temperature dependent changes in sugar content, process quality, invertase activity and tuber respiration rates during LTS and reconditioning of cultivars Russet Burbank, Defender, GemStar Russet and Premier Russet over a 63 day storage period directly following harvest in 2007 and 2008. After sorting, tubers from each of the four cultivars were wound-healed for 12 days at 9 °C during which time basal respiration rates were established. The storage temperature was then rapidly (15 min) dropped to 4 °C (95% RH) for a 30 day LTS period, followed by 3 weeks of reconditioning at 16 °C (95% RH). Tubers from all cultivars were also maintained at 9 °C (95% RH) over the 63 day storage period to serve as controls.

Tubers were sampled at frequent intervals over the 63 day storage period for analysis of process quality, Suc, Glc, Fru and invertase activities. Tubers of each cultivar, ranging in weight from 113 to 340 g per tuber, were blocked for size into four replicates of three (2007) or four (2008) tubers per replicate at each sampling. The tubers were halved longitudinally and the 1.5 mm thick complete central slices from each tuber making up a sample were collectively frozen (−80 °C) and lyophilized. Fry planks (9.5 mm thick × 2.9 cm wide × length of tuber) cut along the apical to basal axis from each of the 12–16 tubers at each sampling were collectively fried in 191 °C vegetable oil for 3.5 min. The color (lightness) of the basal and apical ends of each strip was measured with a Photovolt reflectance meter (Model 577, Photovolt Instruments Inc., Indianapolis, IN) immediately after frying to ascertain process quality. An absolute difference of ≥9 photovolt reflectance units between apical and basal ends of fry planks constituted non-uniform and unacceptable fry color (Driskill et al., 2007). For both long- and short-term studies, fry colors (Photovolt reflectance) were translated to USDA 0 (light) to 4 (dark) color values. Fry colors darker than USDA 2 are unacceptable by industry standards (Driskill et al., 2007).

2.3.1. Carbohydrate analyses

Suc, Glc and Fru concentrations were extracted from 500 mg of lyophilized tissue with 6 mL of 62.5% methanol in triethanolamine–HCl buffer (30 mM, pH 7.0) as described by Knowles et al. (2009). Glc and Fru were determined enzymatically according to Bergmeyer et al. (1974) and Bernt and Bergmeyer (1974). The stoichiometric reduction of NADP as each hexose is converted to 6-phosphogluconate was monitored at A_{340}. Total Glc (free Glc plus that hydrolyzed from Suc) was determined by incubating an aliquot of extract with invertase. Suc was calculated as the difference between moles total Glc and moles free Glc (Bergmeyer and Bernt, 1974). Quantifications were based on standard curves of Glc, Fru and Suc (0.05–2.4 mM).

2.3.2. Invertase activity

Invertase was extracted from 1 g of frozen (−80 °C) tissue with 4 mL HEPES buffer (50 mM, pH 7.5) containing 15 mM $MgCl_2$, 2 mM Na_2EDTA, 2 mM DTT, 10% (v/v) glycerol, 2% (w/v) PVPP and 2 μL protease inhibitor cocktail (2.5 μg mL^{-1} leupeptin, chymostatin, pepstatin and antipain, and 20 μg mL^{-1} 4-aminobenzamidine and benzamidine) (four replicates, four tubers per replicate). Extracts were centrifuged (14,000 × *g*, 25 min, 4 °C) and supernatants desalted at 4 °C through Sephadex G-25 columns. Acid invertase activity was determined colorimetrically by a microplate modification of Brummell et al. (2011) as described in Zommick et al. (2013). The assay entails incubating the extracts with Suc for 30 min at

37 °C, followed by spectrophotometric determination (A_{550}) of the Glc and Fru liberated with Sumner's reagent (Sumner, 1921). Quantitation of Glc was based on a Glc standard curve and acid invertase activity was expressed as nmol Suc hydrolyzed mg^{-1} protein h^{-1} in the presence and absence of its endogenous inhibitor. The inhibitor was removed by vortexing extracts (100 μL aliquots) for 10 min at 23 °C (Zommick et al., 2013). The foamed extracts were immediately assayed for invertase activity; however, no re-association of the inhibitor was apparent within 4 h of vortexing.

2.3.3. Whole tuber respiration

Changes in the respiration rates of tubers of each cultivar were profiled over the wound healing, LTS and reconditioning phases of storage. Tubers of each cultivar were blocked for size (204–253 g per tuber) and sealed in 3.9 L glass chambers (six tubers per chamber, four replicates) equipped with inlet and outlet ports. Airflow to each chamber averaged ca. 83 mL min^{-1} of CO_2-free air. The outflow from each chamber was directed through an LI-6262 infrared gas analyzer (LI-COR, Inc., Lincoln, Nebraska). Carbon dioxide concentrations were recorded every 6 h over the 63 day storage period and respiration rates are reported as mg CO_2 kg^{-1} h^{-1} ±SE.

2.4. Data analysis and presentation

Cultivar-dependent changes in process quality, sugars, invertase activity and respiration rates during wound healing, LTS and reconditioning were highly consistent between storage seasons. Results from the 8 month storage studies are averaged over three storage seasons (2005–07) and the 2008 data are presented as representative for the short term (63 day) storage studies. All data were subjected to analysis of variance with time, temperature and cultivar as independent variables. Data are plotted ±SE. Means are separated by LSD ($P < 0.05$, 0.01 or 0.001) where appropriate.

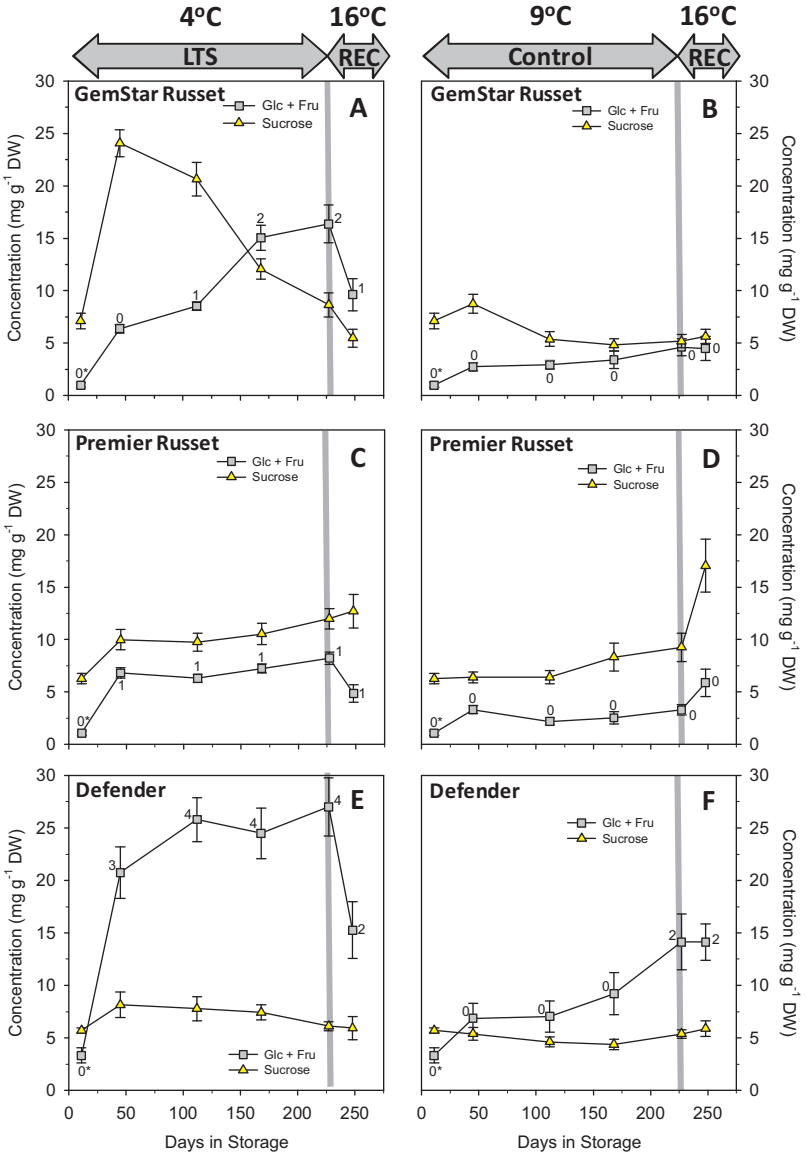

Fig. 1. Changes in reducing sugar (Glc + Fru) and sucrose concentrations in 'Gemstar Russet' 'Premier Russet' and 'Defender' tubers over a 250 day storage period. Tubers were initially wound-healed for 11 days at 12 °C and then stored at 4 °C (LTS, left panel) or 9 °C (Control, right panel) for an additional 217 days. The tubers were reconditioned (REC) for 22 days at 16 °C (from 228 to 250 days). *Numbers are USDA color values of the basal (darkest) ends of French fries. Data are averaged over three storage seasons. Each point represents the average of 36 tubers ±SE.

3. Results

3.1. Sweetening profiles during long-term storage

Tubers were maintained at 4 °C (LTS) or 9 °C (control) for 217 days following a wound healing period (12 °C) to compare changes in sugar content during long-term storage (Fig. 1). Sucrose increased 3.4-fold in 'GemStar Russet' tubers during the initial 34 days of cold storage and then declined concomitant with a rise in RS content (Fig. 1A). While similar trends in Suc and RS were apparent at 9 °C (Fig. 1B), the responses were greatly attenuated, resulting in comparatively low concentrations of both sugars throughout the storage period. 'GemStar Russet' thus exhibited moderate resistance to LTS as total free sugar content was relatively high but the concentration of RS remained sufficiently low for fry colors to remain acceptable (USDA 0–2) by industry standards (Driskill et al., 2007). The inherent resistance of 'Premier Russet' tubers to LTS resulted in low Suc and RS content at 4 °C, though concentrations were higher than in control tubers stored at 9 °C (Fig. 1C, D). This cultivar produced highly acceptable light colored (USDA 0–1) fries throughout the 228 day storage period. In contrast to 'GemStar' and 'Premier Russet', RS concentration in 'Defender' tubers increased 6.2-fold over the initial 34 days at 4 °C and continued to increase marginally through 228 days (Fig. 1E). French fry color of 'Defender' tubers was unacceptably dark (USDA 3) at day 45 (after 34 days at 4 °C) and continued to deteriorate, resulting in USDA 4 colored fries at 112, 169 and 228 days of storage. The increase in RS concentration of 'Defender' tubers was much less at 9 °C (Fig. 1F), resulting in acceptable USDA 0 colored fries through 169 days and USDA 2 fries by 228 days. Reconditioning (22 days at 16 °C) tubers that had been stored for 7 months at 4 °C significantly ($P < 0.01$) lowered RS concentrations and improved process quality, resulting in acceptable USDA 0 ('Premier'), USDA 1 ('GemStar') and USDA 2 ('Defender') colored fries. The LTS of these cultivars was therefore at least partly reversible following 7 months of cold storage.

It was evident from the long-term storage studies that the most rapid and prominent changes in sugar content and process quality occurred within the initial 34 days of storage at 4 °C (Fig. 1). Temperature-induced changes in process quality and carbohydrates were therefore compared among LTS resistant and susceptible cultivars during wound healing (12 days at 9 °C), LTS (30 days at 4 °C) and reconditioning (21 days at 16 °C) in relation to tuber respiration and invertase activity over a 2 month storage period.

3.2. Short-term storage studies

3.2.1. Process quality during low temperature storage (4 °C)

The cultivars for this study represent extremes in LTS-resistance and susceptibly. 'Gemstar Russet' and 'Premier Russet' consistently maintained optimal process quality through 30 days of storage at 4 °C (i.e. from 12 to 42 DAH; Fig. 2). Despite 25% ('GemStar') and 15% ('Premier') decreases in the average Photovolt reflectance of finished fries during LTS (12–42 DAH) (Figs. 3B and 4B), fry colors were uniform and well above the limits for designation as USDA 0 (Fig. 2). In contrast to 'GemStar' and 'Premier Russet', 'Defender' and 'Russet Burbank' tubers were highly susceptible to LTS and their process quality degraded rapidly in cold-storage (Fig. 2). The color of 'Defender' fries began to darken within 2 days at 4 °C and continued to deteriorate progressively, reaching non-uniform USDA 3 color (basal end) after 30 days of LTS (Fig. 5B). Finished fry color of 'Russet Burbank' tubers remained relatively stable for ca. 6 days, then darkened linearly and more rapidly in the basal end, resulting in severe sugar ends (Fig. 6B). When held continually at 9 °C (control) over the 2 month storage period, average finished fry color of cultivars Gemstar Russet, Russet Burbank, and Defender tubers darkened by only 7% (USDA 0) while the color of 'Premier Russet' fries remained constant (data not shown). These cultivar-dependent trends in process quality during low temperature storage were consistent with those from the previous season.

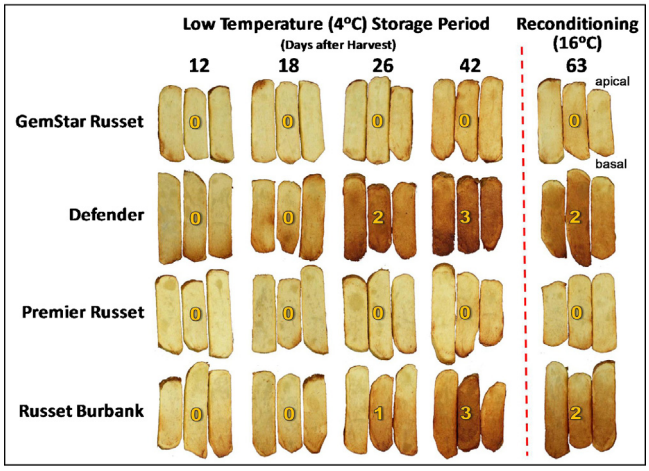

Fig. 2. French fry planks showing changes in process quality during low temperature storage and after reconditioning. Tubers were initially wound-healed for 12 days at 9 °C to establish basal respiration rates (see Figs. 3–6). The temperature was then rapidly lowered to 4 °C for 30 days of LTS (from 12 to 42 DAH) before increasing to 16 °C for 3 weeks to assess reconditioning potential. Each fry plank is from a different tuber and the three planks shown for each treatment were selected to represent the average fry color from a 16-tuber sample. Fry planks are oriented apical end up and basal end (stolon attachment) down. Numbers indicate USDA color classes (0 light to 4 dark) based on average photovolt reflectance values of the basal ends of fries (≥USDA 3 is unacceptable by industry standards).

3.2.2. Low temperature induced sugar accumulation

Changes in Suc and RS concentrations during wound healing, LTS and reconditioning at different temperatures are presented in Figs. 3–6 and a statistical comparison of sugar concentrations among cultivars following each phase of storage is presented in Table 1. While 'Gemstar' and 'Premier Russet' tubers retained

Table 1
Suc and RS (Glc + Fru) concentrations in tubers of LTS-resistant (GemStar Russet and Premier Russet) and susceptible (Defender and Russet Burbank) cultivars following wound-healing (WH), low temperature sweetening (LTS) and reconditioning (REC) periods of storage at different temperatures. Letters indicate mean separation within a column by LSD ($P < 0.05$).

Cultivar	Storage phase (DAH)					
	WH (0–12) 9 °C Sucrose (mg g^{-1} DW)	LTS (12–42) 4 °C	REC (42–63) 16 °C	WH (0–12) 9 °C Glc + Fru (mg g^{-1} DW)	LTS (12–42) 4 °C	REC (42–63) 16 °C
GemStar Russet	5.9a	30.0a	14.8a	2.2a	3.8a	3.0a
Defender	5.7a	12.2b	7.7b	1.9a	22.2b	12.6b
Premier Russet	5.7a	11.2b	5.5b	1.5a	4.2a	1.4a
Russet Burbank	6.0a	13.4b	5.9b	1.8a	17.4b	12.1b

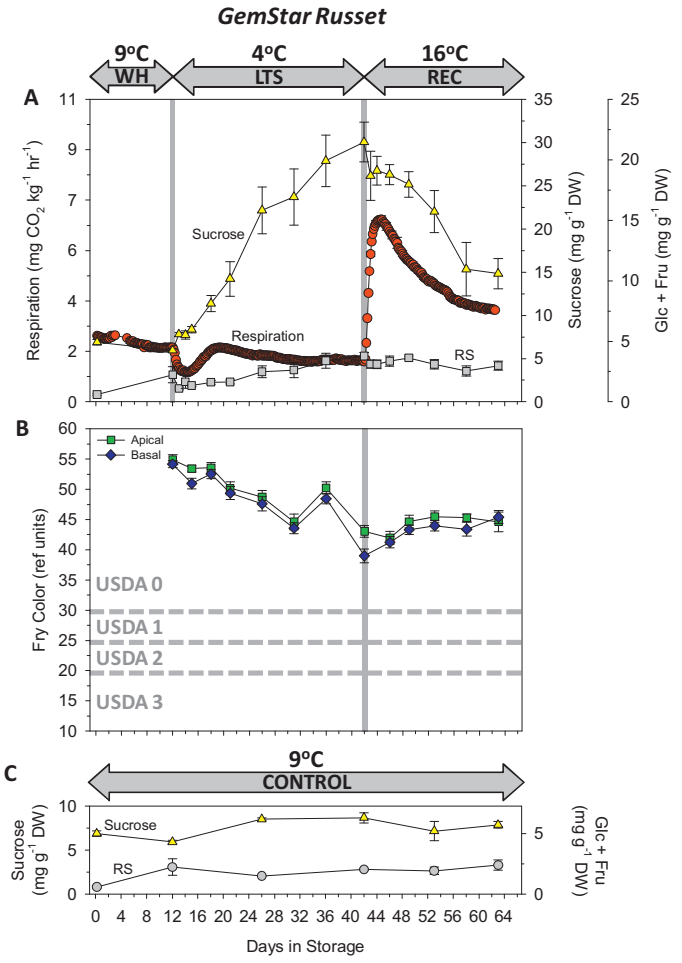

Fig. 3. (A) Changes in respiration rate, sucrose and reducing sugar (RS, Glc + Fru) concentrations of 'GemStar Russet' tubers in response to temperature changes during wound healing (WH), low temperature sweetening (LTS) and reconditioning (REC). Tubers were initially stored for 12 days at 9 °C to establish basal respiration rates during WH. The storage temperature was then rapidly lowered to 4 °C for a 30 day period of LTS (from 12 to 42 days) and then increased to 16 °C for a 21 day period of REC (from 42 to 63 days). (B) Fry color (photovolt reflectance) of the apical and basal ends of fries during LTS and REC. Lower reflectance (ref) values indicate darker colored fries. Dashed lines indicate cutoff reflectance values for USDA 0–3 color ratings. (C) Changes in sucrose and RS in control tubers held continuously at 9 °C (95% RH). *Y*-axes are scaled the same as in (A) for direct comparison of sugar data. For sugars (A and C) and fry color (B) each point is the average of 16 tubers (four replicates of four tubers, ±SE). For respiration rates, each point represents the average of 24 tubers (four replicates of six tubers).

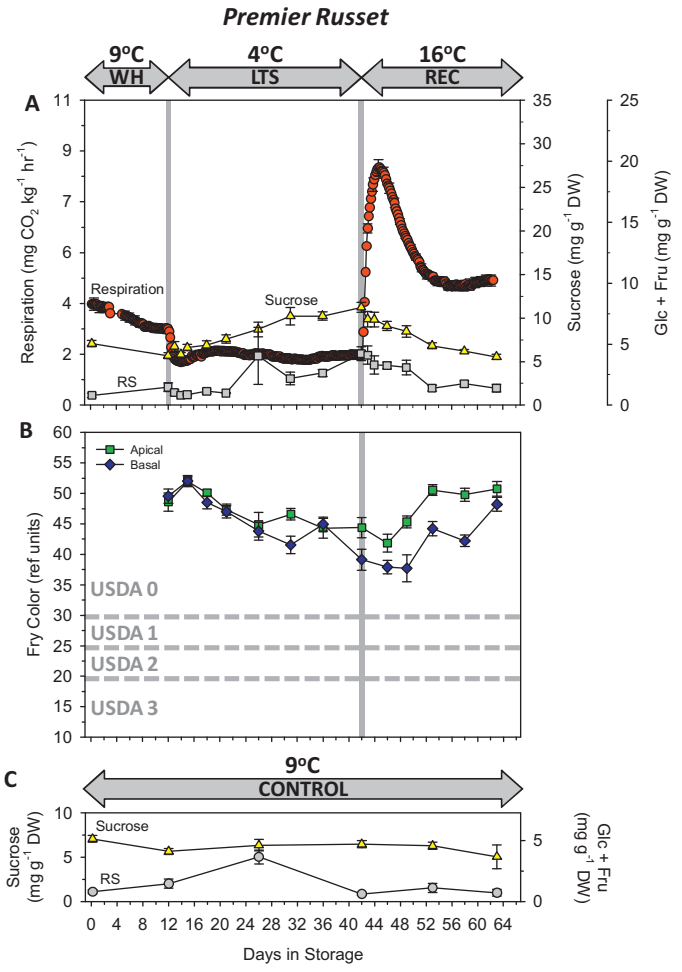

Fig. 4. (A) Changes in respiration rate, sucrose and reducing sugar (RS, Glc + Fru) concentrations of 'Premier Russet' tubers in response to temperature changes during wound healing (WH), low temperature sweetening (LTS) and reconditioning (REC). (B) Fry color (photovolt reflectance) of the apical and basal ends of fries during LTS and REC. (C) Changes in sucrose and RS in control tubers held continuously at 9 °C (95% RH). See Fig. 3 legend for details.

process quality at low temperature, their mechanisms of LTS-resistance varied significantly. Sucrose concentration in 'GemStar Russet' tubers increased 5-fold during 30 days of LTS while RS remained relatively low (Fig. 3A; Table 1). In contrast, Suc and RS concentrations in 'Premier Russet' tubers increased relatively slowly to become 2- and 2.8-fold higher, respectively, after 30 days of LTS (Fig. 4A; Table 1). When held continually at 9 °C, free sugar content in tubers of both cultivars remained relatively constant (Figs. 3C and 4C). The trends in LTS of these cultivars were highly consistent between the two storage seasons.

The decline in process quality of 'Defender' and 'Russet Burbank' tubers paralleled cold-induced RS accumulation (Figs. 5A, B and 6A, B; Table 1). Within the first 2 weeks at 4 °C, RS concentration of 'Defender' tubers increased 10.2-fold while sucrose concentration approximately doubled (Fig. 5A). These trends were also evident from the previous storage season and in both years RS and Suc increased an average of 11.5-fold and 2.0-fold, respectively, over the 30-day LTS period (Table 1; Fig. 5A). Final sugar concentrations were statistically similar between the two sweetening susceptible cultivars after 30 days at 4 °C (Table 1); however, RS and Suc accumulated more gradually in 'Russet Burbank' (Fig. 6A) than in 'Defender' tubers (Fig. 5A), consistent with the initial 7–8 day delays in sweetening and deterioration of process quality in the former (Figs. 2 and 6A, B). Despite these differences in the kinetics of sweetening, Suc concentration increased 2.2-fold and RS 9.7-fold over the 30-day storage period at 4 °C in 'Russet Burbank' tubers (Table 1; Fig. 6A). Control 'Defender' and Russet Burbank' tubers stored constantly at 9 °C for 63 days exhibited no change in Suc levels and a relatively minor increase in RS (Fig. 5C and 6C) compared with that induced by 4 °C (LTS).

Changes in the relative molar proportions of Fru and Glc in tubers during LTS depended on cultivar and correlated with the extent of sweetening. Fru/Glc ratio was equivalent for all cultivars at zero time, averaging 0.62 (Fig. 7). However, cultivar-dependent differences in this ratio became apparent within 1 day of storage at 4 °C and were greatest from 2–6 days of LTS. Fru/Glc ratios increased in 'Defender' and 'Russet Burbank' tubers, remained relatively constant in 'GemStar Russet' tubers, but decreased and then increased in 'Premier Russet' tubers over the 30 day LTS period. 'Premier' and 'Gemstar Russet' consistently exhibited lower Fru/Glc ratios than 'Defender' and 'Russet Burbank'. At 6 days of LTS, Fru/Glc ratios averaged 0.40 ('Premier Russet'), 0.66 ('GemStar

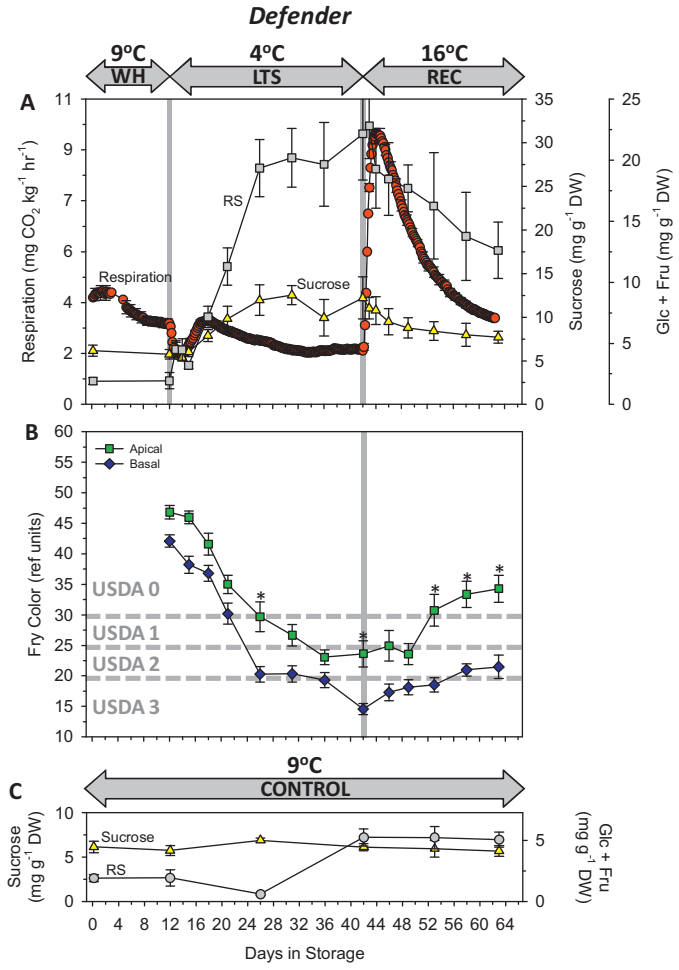

Fig. 5. (A) Changes in respiration rate, sucrose and reducing sugar (RS, Glc + Fru) concentrations of 'Defender' tubers in response to temperature changes during wound healing (WH), low temperature sweetening (LTS) and reconditioning (REC). (B) Fry color (photovolt reflectance) of the apical and basal ends of fries during LTS and REC (*denotes non-uniform fry color). (C) Changes in sucrose and RS in control tubers held continuously at 9 °C (95% RH). See Fig. 3 legend for details.

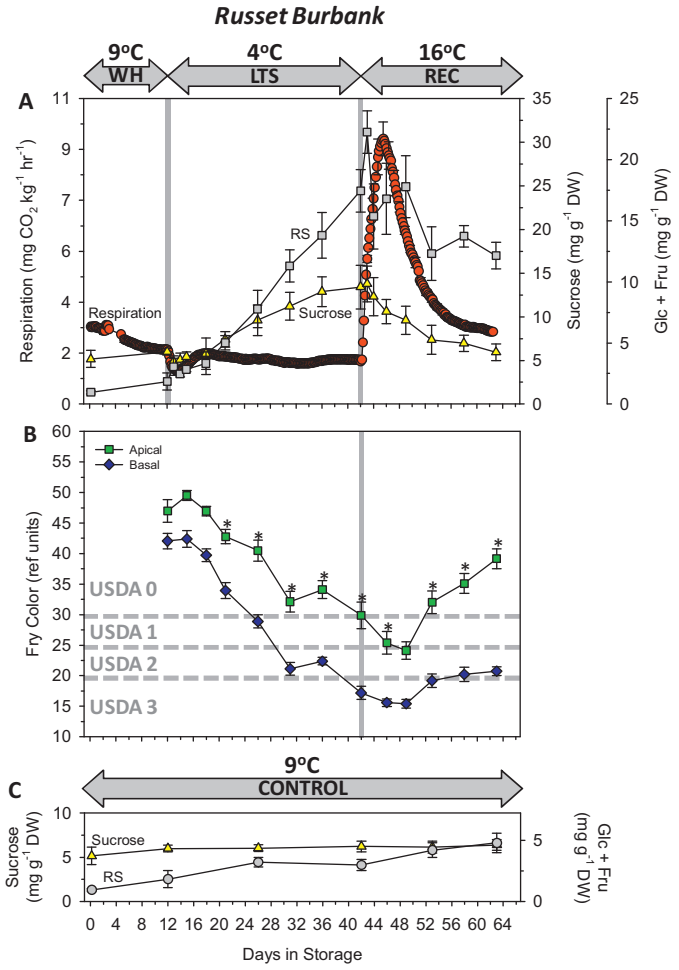

Fig. 6. (A) Changes in respiration rate, sucrose and reducing sugar (RS, Glc + Fru) concentrations of 'Russet Burbank' tubers in response to temperature changes during wound healing (WH), low temperature sweetening (LTS) and reconditioning (REC). (B) Fry color (photovolt reflectance) of the apical and basal ends of fries during LTS and REC (*denotes non-uniform fry color). (C) Changes in sucrose and RS in control tubers held continuously at 9 °C (95% RH). See Fig. 3 legend for details.

Russet'), 0.82 ('Russet Burbank'), and 0.98 ('Defender') (LSD = 0.11, $P < 0.05$). These values correlated ($R^2 = 0.80$, $P < 0.05$) with the RS concentrations following 30 days of storage at 4 °C (Table 1), reflecting genotypic differences in sweetening metabolism and thus the degree of resistance to LTS.

3.2.3. *Effects of reconditioning on process quality and sugar content*

Following 30 days of LTS, storage temperature was rapidly increased to 16 °C for 3 weeks of reconditioning to ascertain the capacity for recovery of processing potential in each cultivar (Figs. 3–6). The Suc and RS concentrations before and after reconditioning are statistically compared in Table 1. Since RS concentrations were relatively low in 'GemStar' and 'Premier Russet' tubers after 30 days at 4 °C (Figs. 3A and 4A; Table 1) and both cultivars were producing highly acceptable USDA 0 fry color (Figs. 2, 3B, and 4B), reconditioning would not normally be necessary. However, storage of tubers at 16 °C for 21 days improved fry color by 10% in 'Gemstar' and 19% in 'Premier Russet' (Figs. 3B and 4B). Consistent with these modest improvements in fry quality, RS concentrations declined to basal levels (i.e. levels prior to LTS) in 'Premier' and by 21% in 'GemStar Russet' during reconditioning (Table 1; Figs. 3A and 4A).

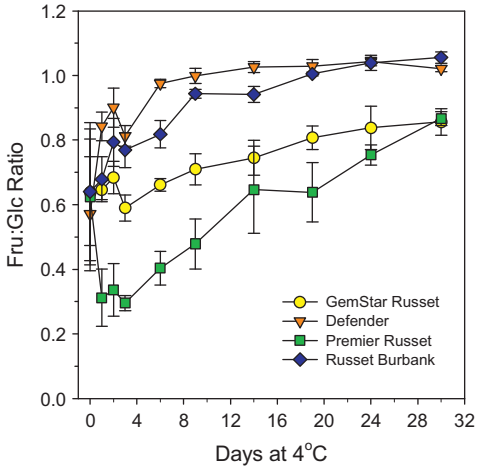

Fig. 7. Cultivar-dependent changes in the ratio of fructose (Fru) to glucose (Glc) in tubers stored for 30 days at 4 °C (95% RH). Tubers were initially wound-healed at 9 °C (95% RH) for 12 days. The temperature was then rapidly lowered to 4 °C for 30 days (LTS phase, 12–42 days, see Figs. 3–6). Each point is the average of 16 tubers (four replicates of four tubers, ±SE).

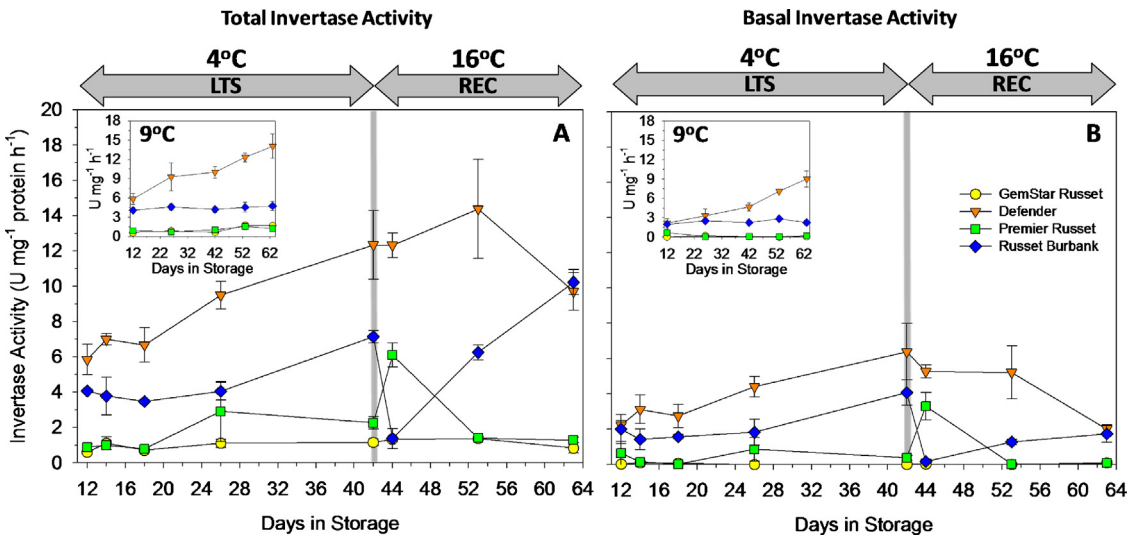

Fig. 8. Cultivar-dependent changes in total (A) (inhibitor absent) and basal (B) (inhibitor present) invertase activities in response to low temperature storage and reconditioning. Tubers were initially wound-healed for 12 days at 9 °C (95% RH). The temperature was then rapidly lowered to 4 °C for 30 days (LTS phase, 12–42 days, see Figs. 3–6) before increasing to 16 °C for 21 days of reconditioning (REC). Control tubers were held continuously at 9 °C after wound-healing (insets). Activity is expressed as nmol sucrose hydrolyzed (units) mg^{-1} protein h^{-1}. Each point is the average of 16 tubers (four replicates of four tubers, ±SE).

Reconditioning 'Defender' and 'Russet Burbank' tubers following 30 days of LTS improved average fry color by 46% and 27%, respectively, although the basal ends of fries in both cultivars remained unacceptably darker than the apical ends (Figs. 2, 5B, and 6B), characterizing irreversible sweetening of the basal ends (i.e. sugar ends). Despite the largest reconditioning-induced decrease in RS in 'Defender' tubers (Table 1), RS remained 6.6-fold higher than at the onset of cold storage (Table 1; Fig. 5A). Exacerbation of sugar-ends during reconditioning at 16 °C undermined any beneficial reduction in RS content in 'Defender' (Fig. 5B) and Russet Burbank' tubers (Fig. 6B).

3.2.4. *Changes in invertase and invertase inhibitor activities during LTS and reconditioning*

Changes in total (no inhibitor) and basal invertase (inhibitor present) activities during low-temperature storage (4 °C), reconditioning (16 °C) and continuous storage at 9 °C depended on cultivar (Fig. 8). Increases in total and basal activities were linearly correlated with increases in RS content at 4 °C (R^2 = 0.86–0.90; $P < 0.001$) in 'Defender', 'Russet Burbank' and 'Premier Russet' tubers. In contrast, total and basal invertase activities were comparatively low and constant during LTS of 'GemStar Russet' tubers (Fig. 8), consistent with the lowest buildup of RS in this cultivar over the 30 day storage period at 4 °C (Fig. 3A; Table 1). When tubers were stored continuously at 9 °C for 63 days (Fig. 8 insets), invertase activities were low and constant in all cultivars except 'Defender'.

'GemStar' and 'Premier Russet' (LTS resistant) tubers were characterized by low and relatively constant total invertase activities and negligible basal activities, the latter resulting from 92% average inhibition of invertase by its inhibitor over the 30 day storage period at 4 °C (Fig. 8A, B). Furthermore, total and basal invertase activities of control tubers held at 9 °C were nearly indistinguishable from the cold-stored tubers (Fig. 8A, B insets), suggesting a nominal effect of temperature on expression of invertase and endogenous inhibitors early in storage. Despite a brief rise in total and basal activities in 'Premier Russet' tubers in the first two days of reconditioning, invertase activities were maintained at relatively low levels through the remainder of the 21 day reconditioning period in both cultivars.

Total invertase activity in 'Defender' tubers was initially 7.8-fold higher than the average of 'Premier' and Gemstar Russet' tubers just prior to LTS and increased 2.1-fold over the 30 day interval of LTS (Fig. 8A) in concert with the rapid increase in RS (Fig. 5A). A similar trend was observed in control (9 °C) tubers, although cold storage induced an 18% higher total activity by 30 days than in tubers stored at 9 °C (Fig. 8A and inset). Total activity in 'Russet Burbank' tubers was likewise initially 5.5-fold higher than the LTS-resistant cultivars at the onset of cold storage; however, unlike with 'Defender', activity remained stable for at least 14 days before increasing in the ensuing 16 days at 4 °C. Total activity remained unchanged for 63 days in control (9 °C) 'Russet Burbank' tubers (Fig. 8A inset). Invertase inhibitor activity (total minus basal) was unaffected by storage temperature and relative inhibition of invertase in 'Russet Burbank' and 'Defender' tubers averaged 52% for the duration of storage at both 4 °C and 9 °C. In response to reconditioning at 16 °C, 'Defender' tubers maintained heightened invertase and inhibitor activities while 'Russet Burbank' tubers exhibited an initial drop in total (5-fold) and basal activities, which then increased linearly (Fig. 8A, B). The initial drop in basal activity in 'Russet Burbank' tubers at the onset of reconditioning is consistent with results of Illeperuma et al. (1998).

3.2.5. *Respiratory acclimation responses (RAR) to changes in temperature*

Whole tuber respiratory profiles were developed for each cultivar during the wound healing, LTS and reconditioning phases of storage (Figs. 3A, 4A, 5A and 6A). As expected, acclimation to the rapid changes in temperature was reflected in altered respiration rates and tubers of each cultivar established new basal rates at each temperature. The RARs to a decrease in temperature (LTS) were characterized by an initial rapid decline to a minimum, increase to a maximum and gradual decline to a new basal rate at the lower temperature (Figs. 3–6). In response to an increase in temperature for reconditioning (REC), respiration rates rose rapidly to a maximum before decreasing to a steady basal rate at the higher temperature.

The temperature changes invoked RARs unique to each cultivar, which reflected their distinctive LTS and reconditioning phenotypes. The RARs are detailed in Fig. 9 with statistical analysis of respiration rates at key points presented in Table 2. The low temperature RARs were quantified as the change in respiration from the lowest rate (trough) to the succeeding highest rate (peak) at 4 °C (Fig. 9A, C; Table 2). The reconditioning RARs were assessed as the relative change in respiration from basal level at 4 °C (day 42) to the peak of the response at 16 °C (Fig. 9B, D; Table 2).

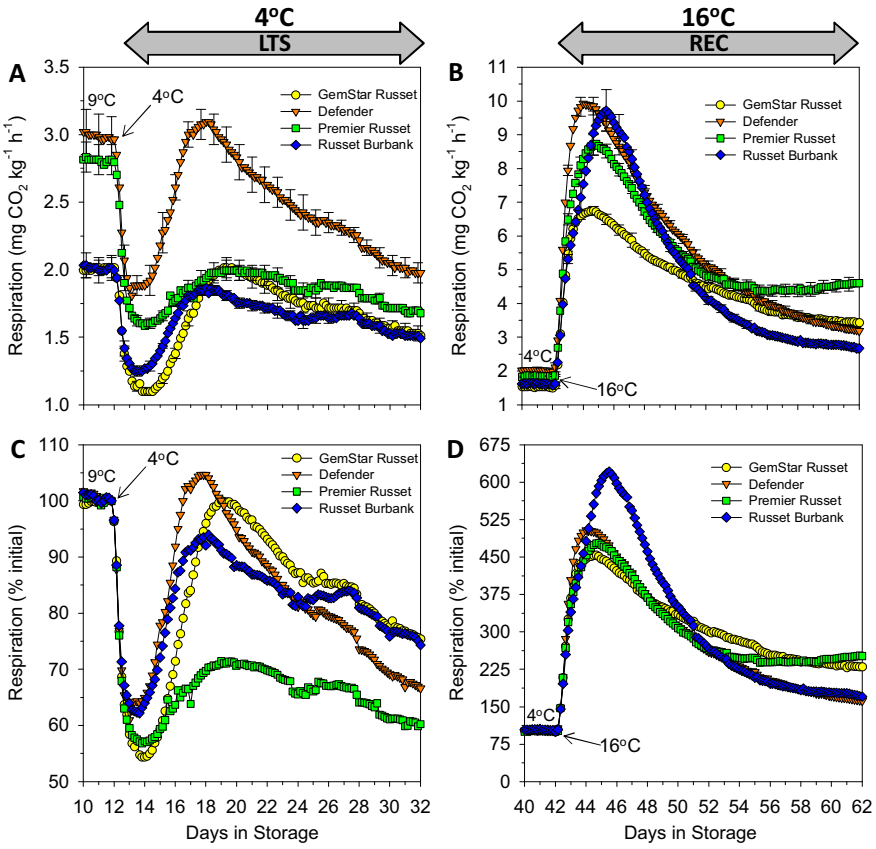

Fig. 9. Respiratory acclimation responses (RAR) of tubers of four cultivars during transitions from 9 °C to 4 °C (low temperature sweetening, LTS) (A and C) and during reconditioning (REC) from 4 °C to 16 °C (B and D). See Fig. 3 legend for details. To directly compare differences in RARs among cultivars, data were normalized to 100% respiration at the transition to LTS at 4 °C (C) and REC at 16 °C (D). Each point is the average of 24 tubers (four replicates of six tubers, ±SE). Respiration rates at key times during acclimation are statistically compared in Table 2.

Averaged over the wound healing period at 9 °C (0–12 DAH) and by the end of 30 days of LTS (day 42), basal respiration rates were consistently higher in 'Defender' and 'Premier Russet' tubers compared with the other cultivars (Fig. 9A, B; Table 2). When challenged with a drop in temperature from 9 to 4 °C, respiration rates fell to a minimum within ca. 2 days followed by a more gradual increase to a maximum by 4–5 days. The immediate decrease in respiration rates when tubers were transferred from 9 to 4 °C averaged 44% for 'GemStar' and 'Premier Russet' tubers compared with 39% for 'Russet Burbank' and 'Defender' tubers (Fig. 9C; Table 2). 'Defender' and 'GemStar Russet' tubers, however, consistently exhibited the most substantial RARs, averaging 61% and 80% increases in respiration in 2007 and 2008, respectively (Table 2; Fig. 9A, C). The RAR of 'Premier Russet' tubers (Δmg CO_2 $kg^{-1}h^{-1}$) was respectively 68% and 55% lower than 'Defender' and 'GemStar Russet' tubers (Table 2; Fig. 9A, C). 'Russet Burbank' tubers exhibited an intermediate cold-induced RAR to 'Premier Russet' (lowest) and the other cultivars, which was estimated at 43% increase in respiration in 2007 and 51% in 2008 (Table 2; Fig. 9A, C). The average reconditioning-induced RAR of cultivars Defender, GemStar Russet and Premier Russet was consistently lower than the RAR for 'Russet Burbank' tubers (Table 2; Fig. 9B, D). These differences are clearly evident in Fig. 9C and D in which respiration rates were normalized to the initial rate at the time temperatures were changed.

4. Discussion

Respiration rates of heterotrophic tissues reflect the energy requirements for maintaining metabolism. "Biosynthesis and all ATP-requiring activities of the cell pull the process of ATP-yielding

Table 2
Tuber respiration rates of LTS-resistant (GemStar Russet and Premier Russet) and susceptible (Defender and Russet Burbank) cultivars during wound-healing (WH), low temperature sweetening (LTS) and reconditioning (REC) periods of storage at different temperatures. Letters indicate mean separation within a column by LSD ($P < 0.05$).

Cultivar	WH (0–12)[a] 9 °C	WH (12) 9 °C	LTS_{trough} (13–14) 4 °C	LTS_{peak} (16–20) 4 °C	LTS RAR (trough to peak)		REC_{base} (42) 16 °C	REC_{peak} (44–46) 16 °C	REC RAR (base to peak)	
					(Peak-trough)	% Increase			(Peak-base)	% Increase
	mg CO_2 kg^{-1} h^{-1}				Δmg CO_2 kg^{-1} h^{-1}		mg CO_2 kg^{-1} h^{-1}		Δmg CO_2 kg^{-1} h^{-1}	
GemStar Russet	2.17d	2.01b	1.09c	2.02b	0.93b	85.3a	1.57c	6.75c	5.18c	330b
Defender	3.42a	2.96a	1.76a	3.09a	1.33a	75.6a	2.11a	9.94a	7.83ab	371b
Premier Russet	3.16b	2.80a	1.59b	2.01b	0.42d	26.4c	1.86b	8.74b	6.88b	370b
Russet Burbank	2.32c	2.00b	1.24c	1.86b	0.63c	50.8b	1.62c	9.76ab	8.13a	502a

[a] Days after harvest.

catabolism; thus, ATP is generated only as fast as it is needed" (Lehninger, 1975). Abiotic and biotic stresses that alter metabolism will therefore often be reflected by altered rates of respiration. As Suc is typically the first free sugar to accumulate in cold-stored potatoes before Glc and Fru (Sowokinos, 1990; Davies and Viola, 1992), the ATP (as UTP) required for synthesis of UDP-Glc and subsequent combination with Fru-6-P by UDP-glucose pyrophosphorylase (UGPase) represents a key sink for ATP in tubers. Isherwood (1973) correlated the estimated ATP generated during the RAR as tubers were moved from 10 °C to 2 °C to almost precisely the energy requirements for Suc biosynthesis in 'King Edward' tubers. Furthermore, manipulating ATP content with uncoupling agents (dinitrophenol; Amir et al., 1977) or controlled atmosphere storage (Solomos and Laties, 1975), modulated decreases or increases in low temperature induced sugar accumulation. In the present study, temperature-dependent changes in carbohydrates and respiration rates in LTS resistant and susceptible cultivars were compared to determine if the RAR to 4 °C is diagnostic of LTS phenotypes.

'Premier Russet' tubers exhibited the most complete LTS resistance, maintaining low concentrations of both Suc and RS (Figs. 1C and 4A) and the lowest RAR to storage at 4 °C (Fig. 9A, C; Table 2). At low temperatures, Suc synthesis in potatoes is under substrate level control (Hill et al., 1996; Krause et al., 1998; Sowokinos et al., 2000). The lack of substantial sugar accumulation in 'Premier Russet' tubers likely reflects restricted availability of UDP-Glc for Suc synthesis, as changes in Suc content of cold-stored tubers closely parallel UDP-Glc concentrations (Hill et al., 1996). The activity of UGPase, responsible for conversion of Glc-1-P to UDG-Glc, was not measured; however, resistance to LTS has been demonstrated in clones which express specific UGPase isozymes (Sowokinos, 2001). The LTS-resistance of 'Premier Russet' tubers can be attenuated by high growing or storage temperatures (Zommick, 2013) and it would be of interest to define the role of UGPase in this response. Further characterization of the mechanism by which heat destabilizes LTS resistance may facilitate the development of genotypes with more robust resistances to both LTS and high temperature stress as well as elucidate a possible role for temperature in contributing to the more subtle seasonal variations observed in the present study.

Moderate LTS resistance in 'GemStar Russet' tubers was characterized by substantial accumulation of Suc and low accumulation of RS during 30 days of storage at 4 °C (Fig. 3A). The unabated synthesis of Suc and greatly suppressed inversion to Glc and Fru were in stark contrast to the sweetening susceptible cultivars (Defender and Russet Burbank), where RS concentrations increased more rapidly than Suc (Figs. 5A and 6A). Consistent with our findings, total activity (inhibitor absent) of acid invertase correlated closely with RS content in cold-stored tubers and the relative contribution of an endogenous inhibitor was highly cultivar dependent (Pressey, 1969; Richardson et al., 1990; Zrenner et al., 1996). A strong correlation between basal activities (inhibitor present) and LTS susceptibilities in 'Defender' and 'Russet Burbank' tubers is consistent with the results of Pressey (1969) in which low inhibitor activity was associated with RS accumulation during LTS. In contrast, invertase activity in 'GemStar Russet' tubers in the presence and absence of inhibitor was extremely low over the initial 30 days of LTS at 4 °C and throughout the 2 month storage period at 9 °C (Fig. 8). Similar trends were described by Matsuuro-Endo et al. (2004) in the sweetening resistant clone 'Inca-no-mezame', but this cultivar exhibited significant Suc accumulation and low RS content in accordance with nominal basal invertase activity through 250 days at 4 °C, while Suc fell and RS gradually accumulated in 'GemStar Russet' tubers over 217 days (Fig. 1A). The increased accumulation of RS, concomitant with a decline in Suc content during long-term storage of 'GemStar' tubers, may thus reflect a reduction in inhibitor activity, similar to the more than 2-fold decline in invertase inhibitor over 210 days at 4 °C observed in 'Pontiac' tubers (Pressey and Shaw, 1966).

The low RS accumulating cultivars, Premier Russet and GemStar Russet, consistently exhibited a significant drop in the ratio of Fru to Glc within 2–6 days of storage at 4 °C relative to the sweetening susceptible cultivars, Defender and Russet Burbank (Fig. 7). Weaver and Timm (1983) correlated this ratio in freshly harvested tubers from 110 day old plants to potential for LTS resistance in storage. In contrast to their results, Fru/Glc ratios of tubers of the cultivars in our study were equivalent (ca. 0.6) under non-sweetening conditions and differences only became apparent after the temperature was lowered from 9 to 4 °C (Fig. 7). In developing and stored tubers, higher concentrations of Glc relative to Fru are modulated by the activity of fructokinases which convert Fru-6-P to Fru-1,6-bisP during glycolysis (Merlo et al., 1993; Davies et al., 2005). Pyrophosphate-linked 6-phosphofructokinase (PPi-PFK) is the most active form of the enzyme in developing and mature tubers (Morrell and ap Reese, 1986) and PPi-PFK was cold-labile in 'Record' tubers (Trevanion and Kruger, 1991), but not in 'Bintji' tubers (Claassen et al., 1991). The role of PFK in dictating the transitory drop in Fru/Glc ratio during the early stages of LTS in 'Premier' and 'GemStar Russet' tubers warrants further investigation. Our results indicate that the initial changes in Fru/Glc ratio upon exposure of tubers to 4 °C are diagnostic of potential for RS accumulation during LTS in these cultivars and when combined with RAR (see below) may effectively identify cold-tolerant germplasm.

The relative ability to improve processing quality through reconditioning at 16 °C depended on cultivar, tuber portion (apical or basal) and the duration of cold storage. When cold-sweetened tubers are exposed to non-sweetening temperatures, Suc is preferentially catabolized to generate Glc for starch biosynthesis (Isherwood, 1976) and increased respiration at the higher temperature (i.e. Q_{10}). 'Defender' tubers exhibited the highest accumulation of RS and darkest fry color in response to LTS; reconditioning therefore had the greatest effect on lowering RS levels and improving finished fry quality in this cultivar (Figs. 2 and 5A, B). The greater reconditioning potentials of 'Defender' and, to a lesser extent, 'Russet Burbank' tubers were further characterized by significantly greater increases in respiration rates during acclimation to 16 °C (Table 2; Fig. 9B, D) and higher invertase inhibitor activities during reconditioning (Fig. 8B), which would potentially favor loss of RS at the higher temperature. Reducing sugar levels were mostly unaffected by reconditioning in 'GemStar' tubers, resulting in little improvement in the already light and uniform fry color following the 30 day LTS period (Fig. 3A, B). However, the accumulation of RS over 217 days of storage at 4 °C was reversible in this cultivar (Fig. 1). Reconditioning 'Premier Russet' tubers lowered the RS levels and improved process color despite highly acceptable USDA 0 fries following the LTS period (Fig. 4A, B). The reduced ability to recondition the basal ends of 'Defender' (Fig. 5B) and 'Russet Burbank' (Fig. 6B) tubers reflects irreversible sweetening associated with the development of sugar ends (Thompson et al., 2008) in these cultivars.

Though the occurrence of a RAR to temperature change has been observed under a variety of storage temperatures in a number of potato cultivars (Isherwood, 1973, 1976; Schippers, 1977; Amir et al., 1977; Workman et al., 1979; Sherman and Ewing, 1983), this study is the first direct comparison of acclimation responses among clones with different LTS resistant phenotypes. Various kinetic parameters of the RAR were assessed in response to decreased storage temperature (e.g., duration of the RAR; slopes during the transient changes in respiration); however, the percent change in respiration from trough to peak (Fig. 9A, C; Table 2) was the best predictor of total sweetening (Suc + Glc + Fru) following 30 days of storage at 4 °C (Table 1). The RAR to 4 °C storage (as % increase in respiration) was consistently highest in 'GemStar' and 'Defender'

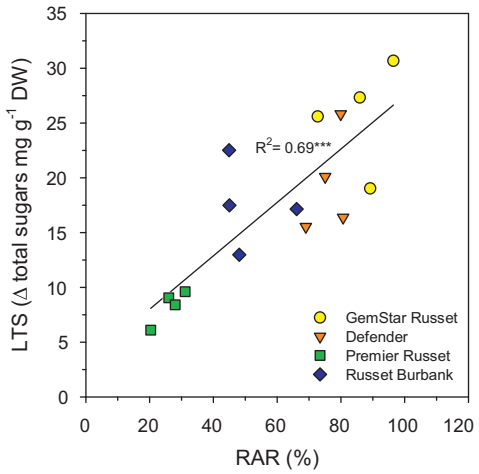

Fig. 10. Correlative relationship between changes in total sugar (sucrose + Glc + Fru) concentration over a 30-day interval of low temperature sweetening at 4 °C and tuber respiratory acclimation response (RAR). RAR is expressed as the percent increase in respiration rate of tubers during the initial week of acclimation to 4 °C (see Table 2 and Fig. 9). ***$P < 0.001$ for correlation coefficient ($n = 16$).

tubers (Table 2), in accordance with rapid starch mobilization and subsequent increases in total sugars (Suc + Glc +Fru) (Table 1) and lowest in highly LTS resistant 'Premier Russet' tubers. The RAR and increase in sugar concentration over the 30 day LTS interval were directly related [mg g^{-1} DW sugars = 3.13 + 0.244(% increase in RAR); $R^2 = 0.69$; $P < 0.001$; $n = 16$] (Fig. 10).

While RAR is indicative of overall LTS, it does not resolve Suc versus RS accumulating phenotypes. On the other hand, Fru/Glc ratio does differentiate these phenotypes during LTS (Fig. 7). For example, the RARs of 'Defender' and 'GemStar Russet' averaged 80% (Table 2) and these cultivars did not differ with respect to the change in total sugars (Suc + Glc + Fru) during LTS (Table 1); however, RS buildup dominated the LTS of 'Defender' while Suc buildup dominated the LTS of 'GemStar' tubers. Suc has no effect on fry color and therefore 'GemStar' tubers process well from low temperature storage (Fig. 2) despite the high level of overall sweetening. Fru/Glc ratios for 'GemStar Russet' tubers after 2–6 days at 4 °C were distinctly intermediate between the LTS susceptible cultivars that accumulated high levels of RS and the LTS resistant 'Premier Russet' that maintained very low levels of both Suc and RS (Fig. 7). Hence, RAR correlates highly with, and is therefore indicative of, the absolute change in total sugars during a 30 day LTS period but it does not resolve high Suc from high RS accumulating phenotypes. RAR and Fru/Glc ratio together indicate LTS phenotypes and further discriminate those that accumulate Suc versus RS when challenged with LTS conditions.

5. Conclusions

In summary, different mechanisms of LTS resistance/susceptibility in cvs. Defender, GemStar Russet, Premier Russet and Russet Burbank were reflected in sweetening and respiratory acclimation responses to temperature change. Cultivar-dependent differences in carbohydrate interconversions and associated metabolism during LTS were indicated by the magnitude of the RAR after transferring tubers from 9 to 4 °C (Fig. 9A, C), likely reflecting the energetics of cold-stress acclimation in relation to sweetening. Loss of process quality in cold-stored 'Defender' and 'Russet Burbank' tubers (Fig. 2) involves catabolism of starch, synthesis of Suc and subsequent inversion to RS, resulting from cold-enhanced invertase activity and generally low inhibitor activity. In 'GemStar Russet' tubers, low invertase in conjunction with high inhibitor activities likely prevented Suc inversion (Figs. 3 and 8), though this resistance mechanism attenuated during long-term storage (Fig. 1A, B). The muted LTS response in 'Premier Russet' tubers (Fig. 4) is indicative of inhibited starch catabolism, which greatly limited the subsequent synthesis of Suc and RS and this was associated with a lower RAR than the other cultivars during the first week at 4 °C (Fig. 9A, C; Table 2). Further analysis of the energetics of carbohydrate metabolism in relation to the respiratory responses of LTS resistant and susceptible cultivars may help to clarify the association between cold-induced sweetening and RAR.

Acknowledgements

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